REMARKS

As a preliminary matter, Applicants respectfully request that the Examiner acknowledge the Supplemental Amendment filed December 18, 2003. The Office Action acknowledges only the Amendment filed October 27, 2003.

The rejection of Claims 1-6, 8-12 and 17-19 under 35 U.S.C. § 102(e) as anticipated by U.S. 6,359,118 (Margolin al), is respectfully traversed.

The present invention relates to a method for the preparation of crosslinked enzyme aggregates (CLEAs), crosslinking agents used in said method, and other methods of using the crosslinking agents.

As described in the specification in Background of the Invention, beginning at paragraph [0002], CLEAs can be regarded as self-supported immobilized enzymes, and which have been used in many fields. In the art, CLEAs are prepared by precipitating the enzymes of interest by a precipitating agent for aggregating the enzymes, which are then crosslinked to one another by a crosslinking agent such as glutaraldehyde.

The present invention is deemed to be an improvement over the above-discussed prior art. As recited in above-amended Claim 1, the invention is a method for the preparation of crosslinked enzyme aggregates, comprising the steps of: A – providing a plurality of enzyme molecules of enzymes, B – aggregating the enzymes in a liquid medium, comprising a precipitating agent, thereof forming aggregated enzymes, C – crosslinking the aggregated enzymes to one another by providing a crosslinking agent in the liquid medium, wherein the crosslinking agent is prepared by reacting a first and a second compound with each other, each having at least two reactive groups, the reactive groups of the first compound being primary amino groups, the reactive groups of the second compound being aldehyde groups.

The goal of the present invention is to crosslink aggregated proteins to one another with a special crosslinker in order to provide sufficient distance between the proteins to be

crosslinked, in order to enable these proteins to keep their biological activity. The crosslinker is chosen such, that it functions as a spacer. By choosing the proper first and second compounds and the proper ratio therebetween, a crosslinking agent of the required length, and therewith the required spacing properties, is obtained.

In contrast thereto, Margolin et al's invention is completely different. Margolin et al discloses crosslinked glycoprotein crystals, such as crosslinked enzyme crystals (CLEC), which are obtained by crosslinking the crystals through one or more carbohydrate moieties on the glycoprotein or through both one or more carbohydrate moieties on the glycoprotein and one or more of the amino acid side chain functional groups in the glycoprotein (paragraph bridging columns 2 and 3, and column 3, lines 35-40). As noted by the Examiner, Margolin et al discloses an embodiment wherein carbohydrate crosslinked glycoprotein crystals are produced by a method including an initial crosslinking reaction in which one or more amino acid side chain functional groups serve as a substrate for a multifunctional crosslinking reagent, such as glutaraldehyde, and the first crosslinking reaction is followed by additional crosslinking reactions in which at least one involves crosslinking through one or more carbohydrate moieties and using, for example, a diamine crosslinking reagent (column 9, lines 1-10).

The Examiner also relies on the disclosure in Margolin et al regarding a second crosslinking procedure involving glutaraldehyde pretreated with, *inter alia*, diaminooctane (sentence bridging columns 25 and 26). But Margolin et al discloses the use of a first (diaminooctane) and a second (glutaraldehyde) compound, as defined in the present invention, to treat the crosslinked enzyme crystals (i.e., already crosslinked by the aminooctane to one another) in order to alter the dissolution thereof (column 25, lines 60-66). Indeed, in the passage relied on by the Examiner, i.e., sentence bridging columns 25 and 26, numerous crosslinking agents can be used for the said second crosslinking agent such as

glutaraldehyde or glutaraldehyde combined with Tris, lysine, or diaminooctane. Since both Tris buffer and lysine contain a single primary amino group, neither would fall under the definition of the first compound according to the present invention.

Margolin et al describes the crosslinking of glycoprotein crystals through one or more carbohydrates moieties with, for example, diaminooctane. Thus, Margolin et al obtains glycoprotein crosslinked enzyme crystals (CLEC's) closely linked to one another. In contrast therewith, the present invention relates to a different crosslinking reaction wherein, as described above, a crosslinker is designed such that a required (functional) distance between the proteins can be obtained. This is not disclosed in Margolin et al.

It should be clear that Margolin et al neither discloses nor suggests a crosslinking agent obtained by **reacting** a diamine crosslinking reagent with, for example, a glutaraldehyde, **prior to** crosslinking their glycoprotein. Indeed, such a technique would appear to frustrate the disclosed goals of Margolin et al. Thus, Margolin et al does not describe the preparation of the crosslinker according to the present invention. According to Margolin et al, both glutaraldehyde and diaminooctane are added in sequence to the already crosslinked substrate (column 27, lines 27-31). Thus, in Margolin et al, a crosslinker as defined to the present invention is not prepared; on the contrary, the glutaraldehyde and diaminooctane are allowed to react with the protein crystals instead of reacting with one another to form a crosslinking agent according to the present invention. Therefore, a crosslinking agent according to the present invention is neither disclosed nor suggested by Margolin et al.

For all the above reasons, it is respectfully requested that the rejection over Margolin al be withdrawn.

The rejection of Claims 1-19 under 35 U.S.C. § 112, second paragraph, is respectfully traversed. Regarding the issue of insufficient antecedent basis in Claim 1, it is now moot in

view of the above-discussed amendment. With regard to Claim 10, while directed to a product, it is claimed in product-by-process terminology. There is nothing indefinite about such a claim, so long as the process steps are definite, because the product obtained thereby is necessarily definite, and its metes and bounds are necessarily ascertainable.

For all the above reasons, it is respectfully requested that this rejection be withdrawn.

The objection to Claim 9 is respectfully traversed. The Examiner finds that it is "preferred" to change "obtainable" to --obtained--. However, such an amendment unnecessarily limits the claims, since the present invention is not limited to crosslinked enzyme aggregates prepared **only** by the method of Claim 1. Note, however, new Claim 20, which employs the term --obtained--.

For all the above reasons, it is respectfully requested that the objection be withdrawn.

Applicants gratefully acknowledge the Examiner's indication of allowability of the subject matter of Claims 7 and 14-16. Nevertheless, Applicants respectfully submit that all of the presently-pending claims in this application are now in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Attorney of Record
Registration No. 24,618

Harris A. Pitlick
Registration No. 38,779

Customer Number
22850

Tel: (703) 413-3000
Fax: (703) 413 -2220
NFO/HAP/cja